Protective effect of ligand-binding proteins against folic acid loss due to photodecomposition

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\begin{abstract}
The B group vitamin known as folates is essential for a variety of physiological processes and plays an important role in the prevention of neural tube defects. However, it decomposes when exposed to UV light. In this study, the response of the synthetic form of folates known as folic acid to UV irradiation in the presence of \(\beta\)-lactoglobulin (\(\beta\)-LG), bovine serum albumin (BSA) and \(\alpha\)-lactalbumin (\(\alpha\)-LA) was investigated using circular dichroism, absorbance and fluorescence spectroscopy. Photodecomposition of folic acid was delayed in the presence of the proteins, which ranked in the order \(\beta\)-LG > BSA > \(\alpha\)-LA in terms of effectiveness. Protein unfolding or decomposition occurred at the same time, due to interaction with folic acid photodecomposition products. The results suggest potential uses of ligand-binding proteins as carriers of water-soluble active compounds for nutraceutical applications.
\end{abstract}

\section{1. Introduction}

Folate is the naturally occurring form of the water-soluble vitamin B found mainly in leafy green vegetables (e.g., spinach and asparagus) and legumes (e.g., beans and peas). Folic acid, a synthetic and oxidised form of folate, is composed of pterin, p-aminobenzoyl and L-glutamic acid (Fig. 1). In vivo, folic acid is reduced on the pteridine ring at positions 5, 6, 7 and 8 to form biologically active tetrahydrofolate \cite{Erbe&Wang,1984}. Tetrahydrofolate and its derivatives act as coenzymes in one-carbon transfer reactions required in the biosynthesis of nucleic acids and proteins. This vitamin is therefore essential for a variety of physiological functions in humans. It plays an important role in the prevention of neural tube defects in infants and might influence the likelihood of developing vascular diseases and some cancers \cite{Lucock,2000}. However, folic acid is sensitive to ultraviolet (UV) light, which causes its decomposition to inactive photoproducts \cite{Off et al.,2005}. Ligand-binding proteins have been reported to bind various molecules of low-molecular weight and can thus be used potentially for the protection and delivery of bioactive molecules \cite{De Wolf&Brett,2005}.

Functioning primarily as a carrier of numerous endogenous and exogenous compounds in the circulatory system, serum albumin is one of the most widely studied ligand-binding proteins \cite{Hu,Liu,Wang,Xiao,Qu,2004}. Bovine and human serum albumins are homologous proteins with 76\% similar tertiary structures. Bovine serum albumin (BSA, 66 kDa) is a large globular protein consisting of about 583 amino acids in a single polypeptide chain. The protein is made up of three homologous domains (I–III), each composed of two sub-domains (A and B). The six sub-domains assemble to form a column-shaped structure \cite{Zhang&Jia,2006}. It is generally accepted that a certain ligand or set of ligands bind specifically to the distinct portions of the protein, namely the two principal binding sites I and II for small heterocyclic or aromatic carboxylic acids, at least two sites for the binding of long-chain fatty acids, and two metal-binding sites \cite{De Wolf&Brett,2005; Hu,Wang,Ou-Yang,Zhou,Liu,2010}.

\(\beta\)-lactoglobulin (\(\beta\)-LG, 18 kDa), the main protein in whey obtained from bovine milk, is a globular protein containing 162 amino acid residues. \(\beta\)-LG folds into a central calyx formed by eight anti-parallel \(\beta\)-strands plus an \(\alpha\)-helix and ninth \(\beta\)-strand located at the outer surface of the \(\beta\)-barrel \cite{Brownlow et al.,1997}. Based on structural similarity, this protein belongs to the lipocalin family,
most members of which are able to bind small hydrophobic molecules in the internal cavity (Kontopidis, Holt, & Sawyer, 2004). Among the external portions of β-LG that have been suggested as ligand-binding sites are the outer surface near Trp19–Arg124, the surface hydrophobic pocket in the groove between the α-helix and the β-barrel, a site near the aperture of the β-barrel and a site at the monomer–monomer interface of the dimer. Due to the existence of multiple binding sites, β-LG can bind a variety of ligands, such as vitamins, fatty acids, and polyphenols (Liang & Subirade, 2008, 2012; Sawyer, Brownlow, Polikarpov, & Wu, 1998).

Alfa-lactalbumin (α-LA, 14 kDa) is the second most prevalent whey protein in bovine milk. This small globular protein is composed of 123 amino acid residues and has a large α-helical domain and a small β-sheet domain, which are divided by a deep cleft and connected by a calcium binding loop. This protein is also able to bind other metal ion (e.g., Mg, Mn, Zn, Na and K) and interact with proteins, peptides, lipids and fatty acids (Cawthern, Narayan, Chaudhuri, Permyakov, & Berliner, 2012). It has been suggested that α-LA might play roles in the transport of hydrophobic lipids, vitamins and metabolites.

BSA, β-LG and α-LA each have distinctive molecular structures and ligand-binding properties. BSA has been reported to interact with folic acid by hydrophilic contact with tryptophan residue at position 132 (Trp132) on the protein surface and by hydrophobic contact with Trp212 inside the molecule (Bourassa, Hasni, & Tajmir-Riahi, 2011). β-LG could bind folic acid at the protein surface, possibly in the groove between the α-helix and the β-barrel, which was proposed to be the reason for the delay in the photodecomposition of folic acid (Liang & Subirade, 2010). However, Vorobey et al. attributed the protective effect of human serum albumin to the photosensitivity by photodecomposition products of folic acid to protein tryptophan instead of folic acid itself (Vorobey, Steindal, Off, Vorobery, & Moan, 2006). In the present study, the impact of BSA, β-LG and α-LA on the photodecomposition of folic acid was investigated using circular dichroism, fluorescence and absorption spectroscopy. It was found that photodecomposition of folic acid was delayed in the presence of the proteins, which ranked in the order β-LG > BSA > α-LA in terms of effectiveness, although the proteins interacted with folic acid with similar affinity. The differing effectiveness may be attributed mainly to interaction of the proteins with folic acid photodecomposition products and to indirect photo-oxidation of the proteins sensitised by folic acid photodecomposition products in a competitive manner. The data gathered from these experiments should provide insight into the possible application of ligand-binding proteins as carriers of water-soluble active compounds in the development of functional foods and pharmaceuticals.

2. Materials and methods

2.1. Materials

β-LG (B variant, Purity ≥ 90%), BSA (≥ 99%), α-LA (Type I from bovine milk, ~85%) and folic acid (~98%) were purchased from Sigma–Aldrich Chemical Company and used without further purification.

2.2. Sample preparation

Stock solutions of β-LG, BSA and α-LA were made by dissolving the proteins in 10 mM phosphate buffer at pH 7.4 to obtain a concentration of 40 μM, determined by measuring absorbance around 278 nm using molar extinction coefficients of 17,600, 43,800 and 28,540 M⁻¹ cm⁻¹, respectively (Collini, D’Alfonso, & Baldini, 2000; Engel, Visser, & Van Mierlo, 2003; Roy, Tripathy, Chatterjee, & Dasgupta, 2010). The protein stock solutions were stored at 4 °C until use. Stock solution of folic acid was prepared freshly for each experiment by dissolving in 10 mM phosphate buffer at pH 7.4 at a concentration of 200 μM. Protein/folic acid mixtures were prepared by adding protein and folic acid stock solutions to phosphate buffer to obtain their respective final concentrations of 20 and 10 μM. All samples were prepared and incubated for about 1 h at room temperature in plastic tubes and covered with aluminium foil prior to analysis.

2.3. Irradiation procedure

Samples in closed 10 mm quartz cuvettes were exposed to ultraviolet light (peak λ = 365 nm, UVA) using an UVL-21 ultraviolet lamp equipped with the mode lamp stand with the fluence rate set at 1 mW cm⁻². Samples were analysed every 10 min for up to 300 min.

2.4. Fluorescence measurements

Steady-state fluorescence was measured using a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Mississauga, ON, Canada) with 5 nm excitation and emission resolutions. Fluorescence emission spectra of proteins and folic acid were recorded at room temperature using excitation wavelengths of 295 and 348 nm, respectively. All measurements were performed using quartz cuvettes of 10-mm optical length. The spectra of the proteins and folic acid were obtained following subtraction of the
buffer/folic acid and buffer/protein backgrounds, respectively, from the raw spectra.

2.5. Absorbance measurements

Absorbance spectra of folic acid were recorded between 240 and 420 nm on a HP 8453 UV–Visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at room temperature. The path length was 1 cm. Buffer and protein backgrounds were subtracted from the raw spectra. Moreover, the turbidity (100-%T) of the folic acid/protein mixtures was determined from the apparent absorbance at 500 nm measured.

2.6. Circular dichroism (CD) measurement

Far-UV CD spectra of proteins were recorded (195–250 nm) at 25°/C using a Jasco J-815 spectropolarimeter (Jasco, Easton, MD, USA). The path lengths are 1 mm for β-LG and α-LA and 0.1 mm for BSA. The ellipticity was recorded at a speed of 100 nm/min, 0.2 nm resolution, 6–8 accumulations, a bandwidth of 1.0 nm.

3. Results

3.1. Influence of proteins on the photosensitivity of folic acid

The fluorescence of the pterin moiety within folic acid molecule has an extremely low quantum yield (<0.005) due to internal quenching (Off et al., 2005; Thomas et al., 2002). Fluorescence intensity is therefore almost negligible as long as the molecule remains intact. As irradiation with UV was prolonged, fluorescence with an emission maximum (λmax) around 450 nm increased markedly (Fig. 2A), indicating photo-induced splitting of folic acid, since both free pterin molecule and its derivatives have relatively higher quantum yields (Thomas et al., 2002). For clarity, only typical spectra are shown. The fluorescence intensity of folic acid at λmax versus time of irradiation with UV exposure in the absence or presence of proteins is shown in Fig. 2B. In the absence of protein, the intensity increased rapidly until 40 min, at which time the first derivative of the intensity versus time plot (inset) peaked. The intensity then increased more slowly and reached a maximum at 110 min, after which it decreased lightly. This indicates folic acid photodecomposition is a complex process possibly involving multi-step interactions.

The effect of the proteins was essentially a slowing of the UV-induced increase in fluorescence intensity. In the case of α-LA, the increase was slower, the most rapid phase spanning the first 80 min, while the maximal intensity, slightly greater than that of folic acid alone, reached at 180 min. In the presence of BSA and β-LG, the increase was even slower, its rapid phase spanning 100 and 140 min, respectively. The maximal intensity in the presence of BSA was similar to that of folic acid alone, but was not reached until 210 min of irradiation with UV exposure. In the presence of β-LG, the maximum fluorescence was not observed until 300 nm of irradiation. These results indicate that proteins can suppress the photodecomposition of folic acid, with the effectiveness of these three ranked in the order β-LG > BSA > α-LA.

3.2. Protein fluorescence and its folic-acid-induced quenching

Protein intrinsic fluorescence due to Trp residues is sensitive to the fluorophore environment. Fig. 3 shows fluorescence emission spectra of BSA, β-LG and α-LA in the absence (a, c and e) and presence (b, d and f) of folic acid in 10 mM phosphate buffer at pH 7.4. The fluorescence excitation wavelength was 348 nm.

Fig. 2. (A) Fluorescence emission spectra of folic acid after various durations of UV irradiation and (B) fluorescence intensity at λmax for folic acid alone (square) and folic acid in the presence of α-LA (circle), BSA (up-triangle) and β-LG (down-triangle) as a function of UV radiation time. Inset: first derivative of the λmax intensity versus irradiation time. The fluorescence excitation wavelength was 348 nm.

Fig. 3. Fluorescence emission spectra of BSA, β-LG and α-LA in the absence (a, c and e) and presence (b, d and f) of folic acid in 10 mM phosphate buffer at pH 7.4.
about 80% and 20% of the total fluorescence (Roy et al., 2010). α-LA has four Trp residues in its hydrophobic core, and Trp26 contributes most of the total fluorescence, with \( \lambda_{\text{max}} \) around 327 nm (Engel et al., 2003). The fluorescence intensity of α-LA is slightly less than that of β-LG, while that of both of these proteins was significantly lower than that of BSA. The fluorescence intensity contributed by tryptophan residues is generally proportional to the residue number and the hydrophobicity of their surrounding environment. Just the opposite was observed for these three proteins, indicating that intra-molecular quenching of Trp fluorescence was greatest for α-LA, followed by β-LG and then BSA.

Extra quenching of fluorescence provides means of studying the binding properties of proteins in solution. The presence of 10 µM folic acid did not affect the \( \lambda_{\text{max}} \) of the three proteins but did decrease their fluorescence intensity (Fig. 3). Normalised relative to the fluorescence intensities of the pure proteins at \( \lambda_{\text{max}} \), folic acid quenched about 29% of the intensities of the three proteins. Considering that the extent of ligand-induced protein fluorescence quenching is correlated with the affinity for the ligand (Thomas et al., 2002), it is suggested that folic acid binds to BSA, β-LG and α-LA with similar affinity. This is consistent with previously reported binding constants of about \( 10^5 \) M\(^{-1} \) for both BSA and β-LG (Bourassa et al., 2011; Liang & Subirade, 2010; Zhang & Jia, 2006). Together with the different influences of the proteins on folic acid photodecomposition as presented above (Fig. 2B), these results indicate that the protective effects of the proteins may be attributed to other factors in addition to the formation of complexes.

3.3. Photo-sensitivity of protein secondary structures in the presence of folic acid

Circular dichroic far-UV spectroscopy is a valuable technique for studying structural transitions of proteins in solution, in particular for characterising secondary structure. Fig. 4A shows far-UV CD spectra of β-LG in the absence and presence of folic acid after UV irradiations of various durations. The spectrum of β-LG shows a typical β-sheet structure with a broad negative minimum around 215 nm (inset). Neither folic acid nor irradiation affected the protein structure. However, the effect of UV irradiation time does appear as an increase in ellipticity and a blue shift at the shorter wavelengths, while producing no effect at \( \lambda > 215 \) nm, in the presence of folic acid (Fig. 4A). After 300 min of irradiation, the spectrum shows a minimum around 202 nm, reflecting a random coil structure. The structural change is similar to that induced by thermal treatment (data not shown) or binding with riboflavin, indicating the unfolding of β-LG (Dalsgaard, Otzen, Nielsen, & Larsen, 2007).

The far-UV CD spectrum of α-LA features two partly overlapping negative bands at 208 and 222 nm (inset in Fig. 4B), which is typical of the α-helical structure. The structure did not change.
in the presence of folic acid or with prolonged UV irradiation. However, the irradiation caused a decrease in the 222-nm ellipticity and a slight increase in the 208-nm ellipticity concomitantly with its blue-shift in the presence of folic acid (Fig. 4B). The trend is similar to that of protein unfolding induced by thermal treatment (Dolgikh et al., 1981). After 300 min of irradiation, the spectrum shows a minimum at 206 nm but with a shoulder around 222 nm, indicating a partial loss of \( \alpha \)-helical content.

Fig. 4C shows far-UV CD spectra of BSA in the absence and presence of folic acid after UV irradiations of various durations. BSA also exhibits a spectral characteristic of the \( \alpha \)-helix (inset). The protein structures did not change in the presence of folic acid while irradiation produced a small increase in spectral intensity. Under irradiation in the presence of FA, the spectral shape otherwise remained the same, with two negative bands around 208 and 222 nm, indicating that the structure of BSA was still predominantly \( \alpha \)-helical. However, prolonging the irradiation caused a gradual loss in the intensity of these two bands. These results suggest a decrease in the total secondary structure content and accordingly a decrease in the number of amino acid residues involved in this structure within the BSA polypeptides.

It has been reported that BSA, \( \beta \)-LG and \( \alpha \)-LA, having multiple ligand-binding sites, can bind a variety of compounds. This makes it possible to bind multiple ligands simultaneously to the same protein molecules to form protein/multi-ligand complexes. For example, there is no competition between folic acid and retinol for interaction with \( \beta \)-LG (Liang & Subirade, 2010). We therefore speculate that the protein unfolding revealed in Fig. 4 may result from interaction of the proteins with products of folic acid photodecomposition, in view of the results shown in Figs. 2 and 3.

### 3.4. Behaviour of folic acid/protein aqueous solutions under irradiation

Turbidity (100-%T) was determined from the apparent absorbance at 500 nm. The critical concentration for the oligomerization of folic acid is 20 \( \mu \)M (Liang & Subirade, 2010). A 10 \( \mu \)M solution was transparent with zero turbidity in 10 mM phosphate buffer at pH 7.4, as shown in Fig. 5A. Throughout the UV irradiation period, the solution appeared to the naked eye to remain transparent, but its turbidity increases linearly over time, reaching a value of about 1.5 at 300 min. These results suggest that photodecomposition products underwent a slight aggregation. The pattern was noticeably different for the folic acid solutions containing \( \alpha \)-LA (Fig. 4B). The turbidity increases gradually and at a faster rate than in the case of folic acid alone, but reached plateau of about 1.3 after 150 min. This suggests that some interactions might occur between the protein and the photodecomposition products of folic acid. In the case of \( \beta \)-LG and BSA (Fig. 5C and D), no significant change was observed until 90 min, after which the turbidity began to increase, reaching values of about 1.3 after 150 min. This suggests that some interactions might occur between the protein and the photodecomposition products of folic acid.
folic acid photodecomposition may be not the same as those occurring with α-LA.

3.5. Photo-sensitivity of the absorption spectra of folic acid alone and in the presence of protein

3.5.1. Absorption spectra of folic acid

Absorption spectroscopy has been used widely to monitor folic acid and its decomposition products. Fig. 6A shows absorption spectra of folic acid as a function of UV irradiation time. The absorption spectrum of folic acid has a weak peak at 348 nm due to the pterin moiety and a strong peak around 281 nm due to both the pterin and p-aminobenzoyl glutamate moieties. The latter produces a shoulder around 300 nm, of which the exact source is unknown (Vorobey et al., 2006). Exposure to UV light causes a decrease in the absorbance around 280 and 300 nm to form a broad band between 265 and 290 nm with a maximum at 275 nm. Changes in the 348 nm band occurring at the same time consist of increasing absorbance with a concomitant red shift beginning after 20 min of exposure to UV light and reaching 360 nm at 60 min followed by blue shift down to 342 nm at 300 min. These results are indicative of folic acid photodecomposition in two steps, which is consistent with previously reported results indicating that the C9–N10 bond of folic acid breaks to yield inactive 6-formylpterin (FPT) and p-aminobenzoylglutamate (PGA), followed by conversion of the former to pterine-6-carboxylic acid (PCA) (Vorobey et al., 2006). FPT has absorption peaks at 278, 310 and 365 nm, while PCA has peaks at 290 and 350 nm, which are associated respectively with the red shift and the subsequent blue shift of the folic acid 348-nm peak. PGA has an absorption peak at 275 nm (Vorobey et al., 2006), which along with the 290 nm peak of PCA may be associated with the broad band observed near 275 nm.

It has been reported that FPT and PCA could sensitize folic acid photodecomposition (Off et al., 2005), and thus contribute to the rapid phase of decomposition observed during the first 40 min of UV irradiation (Fig. 2). However, the sensitisation effect of PCA is less than that of FPT, and sensitisation decreases as folic acid concentration decreases, due to the greater mean distance between the molecules (Off et al., 2005). Both could contribute to the subsequent gradually decreasing rate of decomposition. Furthermore, the slight decrease in the fluorescence intensity beginning after 110 min (Fig. 2B) could be caused by photo-oxidation of PCA, which yields non-pteridinic products (Suárez, Cabrerizo, Lorente, Thomas, & Capparelli, 2000), considering that PCA has a higher quantum yield than FPT. It is not likely due to quenching caused by photodecomposition product aggregation (Fig. 5B), since prolonging irradiation beyond 180 min did not change turbidity (Fig. 5B) but still decreased fluorescence (Fig. 2B) in the presence of α-LA.

3.5.2. Absorption spectra of folic acid/protein mixtures

The absorption spectra of α-LA, β-LG and BSA were not affected by UV irradiation (data not shown). The presence of the proteins did not change the absorption spectrum of folic acid (Fig. 6B–D). As was the case for non-irradiated folic acid, the 348-nm band increased concomitantly with a red shift followed by a blue shift,
but the transition occurred after longer radiation time (100 min) in the presence of α-LA (Fig. 6B). Absorbance around 300 nm began to increase after an initial decrease until 130 min, and a slight decrease was observed in absorbance at 285 nm after 180 min and at 292 nm after 240 min. These changes produced gradually weaker shoulders at 288, 300 and 350 nm as a new strong peak around 263 nm formed concomitantly with an increase in absorbance at λ < 280 nm. In the case of β-LG (Fig. 6C), absorbance at λ < 280 nm increased to form a broad band from 245 nm to 265 nm. A gradual and slight increase was observed in absorbance around 288 nm after 150 min and in absorbance around 300 nm after an initial slight decrease until 180 min. The band around 350 nm increased but did not shift towards longer wavelength over time. BSA (Fig. 6D) caused an increase in absorbance around 300 nm after an initial slight decrease during the 30–120 min irradiation time range and in absorbance around 265, 294 and 350 nm with an upward shift of the entire spectrum. The inflated result has been observed for folic acid entrapped in polymeric micelles (Andrisano et al., 2003) and may be attributed to the increased turbidity (Fig. 5D).

It has been reported that the photodecomposition products of folic acid are more inclined to sensitise the Trp residues in human serum albumin than is folic acid itself (Vorobey et al., 2006). Trp plays a major role in the photo-oxidation pathway in proteins, due to longer wavelength ground state absorption spectrum and higher molar absorption coefficients than Tyr and Phe, and energy transfer from Tyr and Phe in their excited states (Davies & Truscott, 2001; Kerwin & Remmele, 2007). Indirect photo-oxidation can result in protein unfolding and possibly protein fragmentation (Tattison, Rahmanto, & Davies, 2012). A strong band at 260 nm and a weak band at 320 nm reported at the expense of absorption at 280 nm, attributed to kynurenine and its derivatives, photodecomposition products of Trp (Belcher, Sansone, Fernandez, Haskins, & Brancaloe, 2009; Fujimori, 1982). The spectral change due to UV radiation may therefore result from both folic acid decomposition and protein oxidation. The changes can be observed more clearly in the difference spectra obtained by subtracting the folic acid background (inset in Fig. 6). It is apparent that the change in the spectra thus obtained is less than that observed for folic acid alone (e.g., in Fig. 6A), which provides additional support for the protective effect of protein against the photodecomposition of folic acid as shown in Fig. 2.

4. Discussion on the mechanism by which ligand-binding proteins protect folic acid against photodecomposition

Folic acid is sensitive to UV light and decomposes in multiple steps beginning with breakage of the C9–N10 bond to form FPT and PGA, followed by conversion of the former to PCA, which then yields non-pteridinic products. Both FPT and PCA can sensitise folic acid photodecomposition (Off et al., 2005). However, photodecomposition can be delayed by ligand-binding proteins such as α-LA, BSA and β-LG, which interact not only with folic acid but also with its photodecomposition products. The formation of complexes with protein molecules decreases the photosensitivities of free folic acid essentially by decreasing the effective concentration of all reagents involved in its photodecomposition. The differences in the protection provided by the proteins are attributable to the nature of their interactions with folic acid photodecomposition products. Proteins such as α-LA, BSA and β-LG are not themselves sensitive to UV light, but they do unfold or degrade (Fig. 4) by interacting with folic acid photodecomposition products, which can sensitise the proteins in a competitive manner. The photolability of tryptophan residues depends mainly on their location within the protein molecules (Pigault & Gerard, 1984). Exposed Trp61 in β-LG and Trp134 in BSA are more labile than tryptophan residues in α-LA, which are all located in the hydrophobic interior. The two proteins thus provide better protection of folic acid against photodecomposition (Fig. 2). Trp fluorescence quenching inside proteins ranked as α-LA > β-LG > BSA. The energy transfer can also reduce photo-oxidation of the proteins. BSA is the most vulnerable to photo-oxidation while α-LA is the least vulnerable. The result is BSA degradation, β-LG unfolding and α-LA partial unfolding (Fig. 4).

It has been reported that BSA and β-LG, having multiple ligand-binding sites, can bind a variety of compounds. UV-irradiated folic acid solutions containing these proteins displayed behaviour that different from that of solutions containing folic acid alone or folic acid with α-LA (Fig. 4). Prior to 90 min of irradiation, the turbidity of BSA/folic acid and β-LG/folic acid solutions did not increase as that of solutions of folic acid alone did (Fig. 4A) due to photodecomposition (Fig. 3), indicating that folic acid photodecomposition products are bound to BSA and β-LG in solution. In Fig. 5C and D, the 348-nm band did not shift towards longer wavelengths associated with FPT formation, suggesting the formation of complexes of photodecomposition products with BSA and β-LG, which transfers FPT to a more hydrophobic environment and draws its absorption band towards a shorter wavelength. The formation of complexes with proteins may thus reduce contact between folic acid and its photodecomposition products to some extent, thus reducing the sensitisation of folic acid to photodecomposition. In comparison with BSA, the more effective protection provided by β-LG suggests that this protein binds larger amounts of folic acid photodecomposition photoproducts.

5. Conclusions

Folic acid is sensitive to decomposition by exposure to UV light while α-LA, BSA and β-LG are sensitive to structural change by exposure to UV light in the presence of folic acid. These ligand-binding proteins can interact not only with folic acid but also with its photodecomposition products, leading to unfolding or degradation of the proteins. These proteins can also suppress the photodecomposition of folic acid, and rank in the order β-LG > BSA > α-LA in terms of effectiveness. The differing effectiveness may be attributed mainly to interaction of the proteins with folic acid photodecomposition products and to indirect photo-oxidation of the proteins sensitised by folic acid photodecomposition products in a competitive manner. These results suggest that ligand-binding proteins have potential as carriers of water-soluble active compounds.

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